

# Pathology of the equine salpinx

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## Summary

Equine uterine tubes were evaluated macroscopically (n=100) and microscopically (n=76). Macroscopic lesions occurred as infundibular adhesions and infundibular cysts. Microscopically, the most common findings were intraepithelial cysts within the ampulla, slight, multifocal epithelial and subepithelial infiltration with lymphocytes and the occurrence of collagen masses within the lumen of the ampullary-isthmus junction.

In the majority of the uterine tubes epithelial cells showed an extraordinary coexpression of desmin and cytokeratin. The pathological significance of this coexpression is questionable and might be attributed to the physiologic hormonal activity during cycle and pregnancy respectively.

For the first time chlamydial antigen was demonstrated in the equine salpinx by means of an immunohistologic technique. In five of twenty mares a positive staining for *Chlamydia psittaci*-antibody was evident in some epithelial cells of the isthmus and to a lesser extent in those of the ampulla. These mares showed a multifocal cytoplasmic eosinophilia of the uterine tube epithelium and a slight to moderate endometrosis of the endometrium. In two mares detection of chlamydial antigen was associated with a slight chronic salpingitis and in two other mares with a slight chronic endometritis.

**Keywords:** equine, salpingitis, chlamydia, desmin, immunohistology

## Pathologie der equinen Salpinx

Eileiter von Stuten wurden makroskopisch (n=100) und mikroskopisch (n=76) untersucht. Als makroskopische Veränderungen lagen infundibuläre Adhäsionen und infundibuläre Zysten vor. Die häufigsten mikroskopischen Befunde waren intraepitheliale Zysten, multifokale Infiltrationen von Lymphozyten sowie luminal vorkommende kollagene Massen im Bereich des Übergangs von der Ampulla in den Isthmus.

Bei der immunhistologischen Untersuchung zeigten die Epithelzellen der meisten Eileiterepithelien eine außergewöhnliche Koexpression von Desmin und Zytokeratin. Die Bedeutung dieser Koexpression ist fraglich, ursächlich könnten physiologische Veränderungen des Hormonstatus im Verlauf des Zyklus beziehungsweise der Gravidität verantwortlich sein.

Mit Hilfe immunhistologischer Verfahren wurde erstmalig Chlamydien-Antigen in der equinen Salpinx nachgewiesen. In fünf von zwanzig untersuchten Stuten konnte Chlamydien-Antigen in den Epithelzellen des Isthmus und in einem geringeren Maß in der Ampulla dargestellt werden. Diese Stuten zeigten eine multifokale zytoplasmatische Eosinophilie des Eileiterepithels und eine gering- bis mittelgradige Endometrose des Endometriums. Der Nachweis von Chlamydien-Antigen war bei zwei Stuten mit einer geringgradigen chronischen Salpingitis und bei zwei weiteren Stuten mit einer geringgradigen chronischen Endometritis verbunden.

**Schlüsselwörter:** Pferd, Salpingitis, Chlamydien, Desmin, Immunhistologie

## Introduction

The mammalian uterine tube serves as a conduit from the ovary to the uterus providing a satisfactory environment for final maturation of the oocyte, sperm transport, sperm capacitation, fertilisation and early embryonic development.

Due to the unique conditions of the equine salpinx, the environment of the uterine tube is a known intrinsic factor of possible causes for embryonic loss in mares. These conditions are a prolonged passage of the equine embryo, resulting in a more advanced stage of development of the embryo, retention of unfertilised oocytes and the selective passage of embryos (Ball, 1988).

There is extensive literature on the pathology of the equine endometrium relating to reduced fertility, but in only very few studies has the influence of alterations of the equine salpinx been examined.

Pathological lesions of the uterine tube play an important role in other domestic animals such as cows (Kessy *et al.*, 1985) and sows (Wilson *et al.*, 1949). Tube-associated infertility in women is frequently caused by *Chlamydia*-in-

duced salpingitis. A similar etiology of equine salpingitis has not yet been reported.

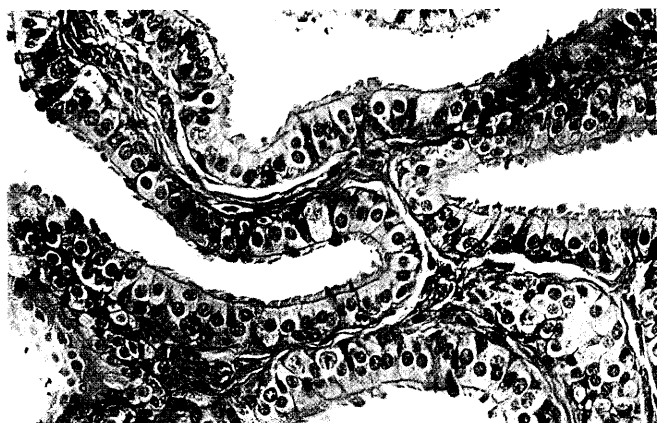
The object of this study was

- to characterise pathological findings of the equine uterine tube by light microscopy, supported by immunohistological examination,
- to determine the presence of chlamydial antigen in the equine salpinx by immunohistological technique and
- to evaluate possible relations between inflammatory processes of the uterine tubes and the endometrium.

The uterine tube of the mare, usually 20 to 30 cm in length and 1.5 to 3.0 mm in diameter, can be distinguished in three parts: the infundibulum, the ampulla and the isthmus running into the uterus (Threlfall, 1995).

The epithelium of the mucosa consists of two cell types: ciliated and non-ciliated cells (Fig. 1). The ciliated cells play an important role in the transport of the oocyte, while most of the non-ciliated epithelial cells represent secretory cells producing and releasing proteins as well as glycoproteins

into the lumen. A third resident cell type, the peg cell, is likely to be a morphologic variant of the secretory cell (Liebich et al., 1999).

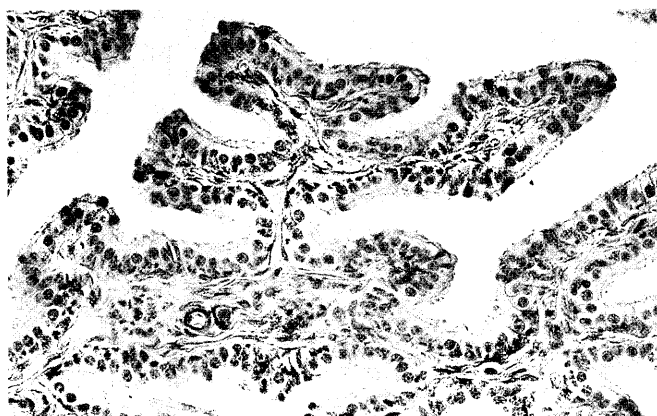


**Fig. 1:** Normal mucosa of the ampulla, 13-year-old mare, luteal phase: The epithelium consist of ciliated and secretory cells, single lymphocytes are found between the epithelial cells or in the lamina propria. (PAS-alcianblue stain, magnification 125x).

Unveränderte Mukosa der Ampulla, 13jährige Stute, Gelbkörperphase: Das Epithel besteht aus Zilienzellen und sekretorische Zellen, einzelne Lymphozyten liegen zwischen den Epithelzellen oder in der Lamina propria. (PAS-Alcianblau-Färbung, Vergrößerung 125x)

## Material and methods

From summer 1996 to winter 1998 a total of 100 genital tracts from mares of different age and breed was collected at the Institute of Veterinary Pathology of the Leipzig University and at slaughterhouses and fixed in formalin. The



**Fig. 2:** Normal mucosa of the ampulla, 3-year-old, pregnant mare (third month): In several ciliated epithelial cells and in the smooth muscle cells of the vessels an intracytoplasmic expression of desmin is evident. The secretory cells of the epithelium are not labelled. (Immunohistology, desmin, Nomarski-interference-contrast, magnification 125 x).

Unveränderte Mukosa der Ampulla, 3jährige, tragende Stute (3. Monat): In epithelialen Zilienzellen und in den glatten Muskelzellen der Gefäße ist eine intrazytoplasmatische Expression von Desmin nachweisbar. Die sekretorischen Zellen reagieren negativ. (Immunohistologie, Desmin, Nomarski-Interferenz-Kontrast, Gerätevergrößerung 125 x)

cycle stage was ascertained by examination of the ovaries and the uterine tubes were first evaluated for gross abnormalities and lesions.

One half of the uterine tubes of 76 mares was removed by longitudinal section from the transition of the infundibulum into the ampulla to the tip of the uterine horn. Additionally, a part of the corpus uteri was excised. The specimens were routinely embedded in paraffin. The sections were stained, using hematoxylin-eosin-, periodic acid-Schiff (PAS)-alcianblue-, toluidine blue-, picro-sirius red- and Papanicolaou staining.

The endometria were classified in categories according to Kenney and Doig (1986).

For immunohistological studies, the tissues were stained for CD 3, IgA, IgG and IgM and for the intermediate filaments desmin, cytokeratin and vimentin by using the peroxidase-anti-peroxidase-technique.

The specimens were investigated by enzyme histochemistry for CLAE (chloracetate esterase).

The uterine tubes of 20 mares were stained for the expression of chlamydial antigen using a Chlamydia psittaci-specific monoclonal antibody.

## Results

### Expression of intermediate filaments

Stromal cells and vascular endothelia are stained positive for vimentin. The unstriated muscles and the smooth muscle cells of the vessels show an expression of desmin and to a lesser extent of vimentin.

Epithelial cells exhibit expression of cytokeratin and in most of the mares unexpectedly a coexpression of desmin is revealed. In these cases desmin appears to be present especially within the ciliated cells (Fig. 2).

The staining for desmin in the ampulla, compared with the isthmus, is more remarkable, varies in intensity and occurs as small spots either in the basal or the apical cytoplasm.

In only two of the 26 mares examined is the epithelial expression of desmin undetectable. Both mares are found in oestrus, in the absence of other parameters such as age or pathological alterations. In seven mares some epithelial cells of the ampulla with a very slight staining are observed. The epithelium of 13 mares is stained slight to moderate and the epithelium of four mares is strongly stained for desmin revealing two pregnancies.

### Pathology

#### Infundibular adhesions

In nearly half of the mares examined, thin fibres of connective tissue are found, mostly bilaterally, linking fimbriae and ovary. The adhesions are observed as thin strands of fibrous tissue, sometimes associated with blood clots, between infundibulum and ovary, mesovarium, mesosalpinx or the uterus (Fig. 3). In no case however is the infundibulum obstructed by the adhesions.

### Infundibular cysts

More than one third of the mares (n=37) has cysts, observed macroscopically in the fimbriated portion of the uterine tube (fig. 4). The cysts occur unilaterally (n=23) and bilaterally (n=14).



**Fig. 3:** Infundibular adhesions: Thin fibres of connective tissue linking the infundibulum and the mesosalpinx to the Ligamentum ovarii proprium.

Adhäsionen des Infundibulums: Zwischen Infundibulum, Mesosalpinx und Ligamentum ovarii proprium befinden sich dünne Bindegewebsfäden.

The diameter of the predominantly small cysts varies from a few millimetres up to one centimetre, only very few are larger than two centimetres. The uterine tubes concerned either show a single cyst (n=32) or a series of two to four adjacent frequently small cysts (n=19).

Their lumina contain a transparent fluid. The epithelial cells of the cysts are flattened or cuboidal with hyperchromatic elongated or round nuclei. The appearance of some epithelial cells lining the cystic lumen is similar to those of the uterine tube. Some cells have few short cilia on their surface, whereas others resemble secretory cells.

### Intraepithelial cysts

Intraepithelial cysts are found frequently in the ampulla but not in the isthmus. The cysts are most often located within the epithelium along the sides and the bases of the mucosal folds. Their diameter varies from 10 to 100 micrometer and the type of cell lining differs. Some of them are flattened with slender nuclei and orient perpendicular to the luminal space, others are cuboid with round nuclei. Occasionally cilia project into the cystic lumen. Some cysts contain material that is stained light blue with PAS-alcianblue (Fig. 5).

13 of 66 mares have no intraepithelial cysts. In 9 mares only one or two single intraepithelial cysts in the total of the longitudinal section are detectable.

The marked presence of intraepithelial cysts is found in most of the mares (n=44) and classified into three degrees:

- slight (+) – up to five cysts per 5 mm (n=30),
- moderate (++) – six to fifteen cysts per 5 mm (n=11) and
- severe (+++) – more than fifteen cysts per 5 mm (n=3).

Frequently the appearance of intraepithelial cysts is observed bilaterally (n=35) and less often unilaterally (n=7).

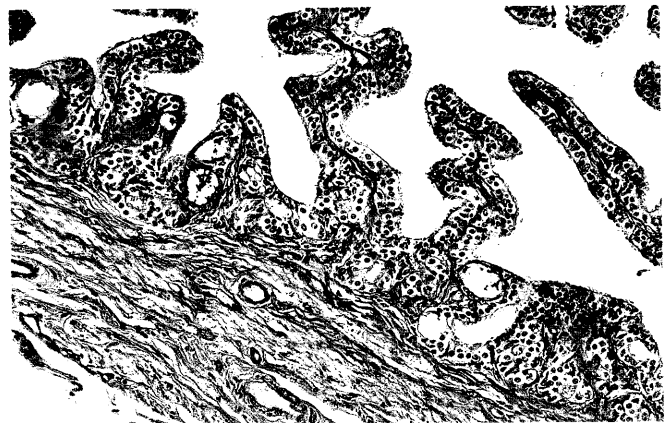


**Fig. 4:** An infundibular cyst with a diameter of 1.0 x 1.3 x 0.9 cm is located at the fimbriae (fixed in formalin; the uterine tube is partially opened).

An den Fimbrien des Infundibulums befindet sich eine Zyste mit einem Durchmesser von 1,0 x 1,3 x 0,9 cm (in Formalin fixiert, partiell eröffnete Eileiter).

The epithelial expression of desmin is most intense in those mares classified as moderate (++) or severe (+++) with regard to the intraepithelial cysts.

It is striking that five of 13 mares of known age with moderate (++) or severe (+++) degree of intraepithelial cysts are three years old. Two thirds of the mares are younger than eight years, one young mare is pregnant.



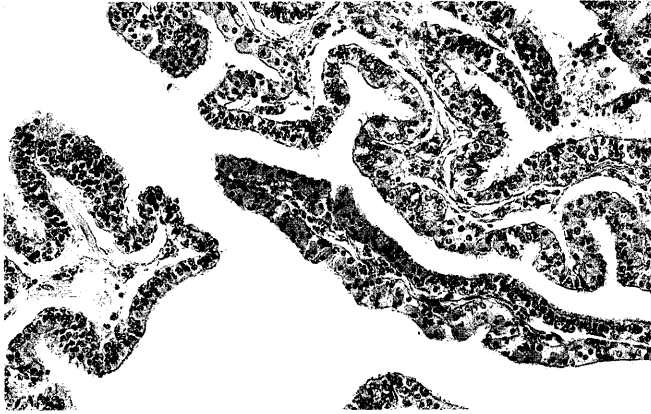
**Fig. 5:** Ampulla, 3-year-old, pregnant mare (third month): Intraepithelial cysts are located at the bases of the mucosal folds, their lumina contain light blue stained material. (PAS-alcianblue stain magnification 62.5 x)

Ampulla, 3jährige, tragende Stute (3. Monat): Intraepitheliale Zysten sind an der Basis der Schleimhautfalten lokalisiert, ihr Lumen beinhaltet leuchtend blau gefärbtes Material. (PAS-Alcianblau-Färbung, Gerätevergrößerung 62,5 x)

### Degeneration of epithelial cells

In five mares a cytoplasmic eosinophilia affecting several groups of epithelial cells identified by HE staining is remarkable. These epithelial cells show an alteration of the epithelial structure as well. They are increased in size, rounded, with a small round hyperchromatic nucleus and a vacuolated cytoplasm giving the cells a foamy appearance (Fig. 6). The cytoplasm of these epithelia is stained light green-grey and their nuclei dark grey in the Papanicolaou stain.

The epithelium frequently seems to be stratified. Only in one mare is cytoplasmic eosinophilia not associated with alterations of the epithelial structure.

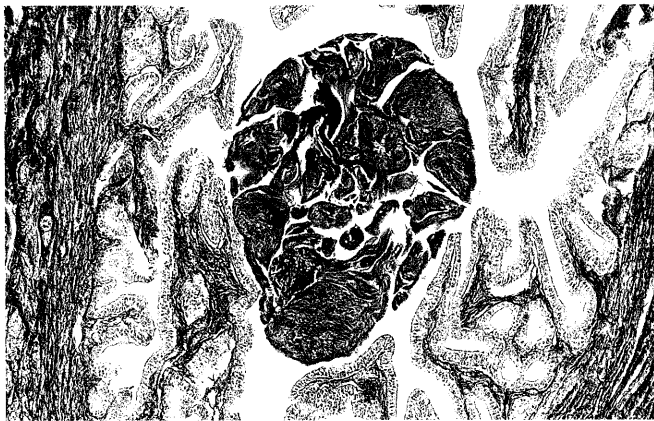


**Fig. 6:** Ampulla, 21-year-old mare: Cytoplasmic eosinophilia of degenerated epithelial cells. (H.-E. stain, magnification 62.5 x)

Ampulla, 21jährige Stute: Zytoplasmatische Eosinophilie der degenerierten Epithelzellen. (H.-E.-Färbung, Gerätevergrößerung 62,5 x)

#### Collagen masses

In half of the mares uterine tubes are found to contain eosinophilic masses of varying size, mostly unilaterally. The masses are located near the ampullary-isthmic junction consisting of swirling bundles of fibres. They are not connected to the epithelium and frequently distend the lumen. The bundles of fibres are stained red with picro-sirius red (Fig. 7) and blue-violet with PAS-alcianblue. These results strongly suggest that the masses consist of collagen fibres.



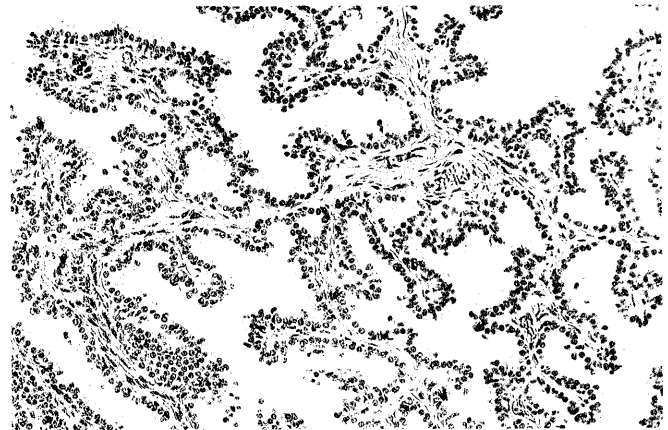
**Fig. 7:** Ampullary-isthmic junction, 18-year-old mare: Collagen masses are located within the lumen and consist of swirling bundles of fibres. There is no attachment to the epithelium. (Picro-Sirius Red stain, magnification 31.25 x)

Übergang der Ampulla in den Isthmus, 18jährige Stute: Kollagene Massen sind im Lumen lokalisiert und bestehen aus wirbeligen Faserbündeln, die nicht mit dem Epithel verbunden sind. (Pikrosirius-rot Färbung, Vergrößerung 31,25 x)

#### Salpingitis

In the ampulla as well as in the isthmus of the unaffected uterine tubes a few lymphoid cells are located between the

basal epithelia and a comparable number of cells are scattered throughout the lamina propria. These small and oval or round cells with dark nuclei and scanty hypochromatic cytoplasm are stained positive for CD 3 surface antigen, thereby characterising those cells as T lymphocytes (Fig. 8). Inflammatory processes at the entrance of the ampulla are obvious in one third of the mares (n=27, unilateral: n=12, bilateral: n=15). Most of these mares (n=16) show a slight multifocal or diffuse infiltration with lymphocytes in the ampullar mucosa sometimes associated with plasma cells, hyperaemia or oedema of the mucosa. Plasma cells expressing IgA are the most common cell type in the lamina propria. Occasionally plasma cells of IgG and rarely of IgM subtype are identified. Moderate to severe salpingitis with infiltration of lymphocytes was found less often (n=6).



**Fig. 8:** Normal mucosa of the ampulla, 3-year-old, pregnant mare (third month): T lymphocytes (brown) in the epithelium and in the lamina propria. (Immunohistology, CD 3 surface antigen, Nomarski-interference-contrast, magnification 62.5 x)

Unveränderte Mukosa der Ampulla, 3jährige, tragende Stute (3. Monat): T-Lymphozyten (braun) im Epithel und in der Lamina propria. (Immunhistologie, CD 3 Oberflächenantigen, Nomarski-Interferenz-Kontrast, Gerätevergrößerung 62,5 x).

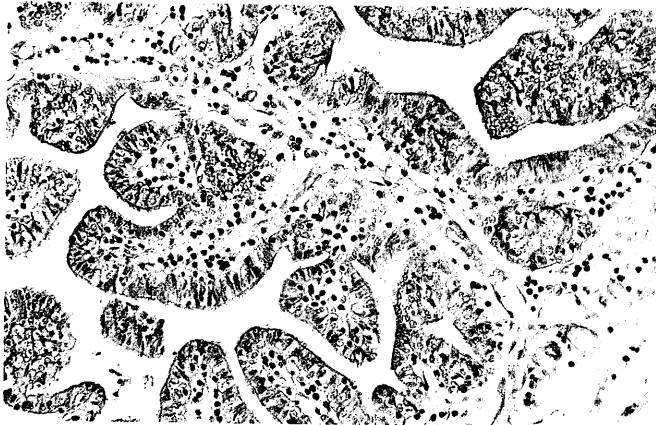
In rare cases (n=5) a moderate to severe infiltration with neutrophilic granulocytes is observed and interpreted as indicative for an exudative salpingitis (Fig.9). In three cases some eosinophilic granulocytes are detectable in the lamina propria.

Neither slight nor moderate mononuclear infiltrations are associated with mucosal damage as observed by light microscopy. Severe infiltration of lymphocytes and neutrophilic granulocytes however causes destruction of epithelial cells.

The unaffected isthmus, compared to the ampulla, shows a lower amount of T lymphocytes (CD 3 positive cells). Inflammation of the isthmus is diagnosed in 12 mares characterised by a slight (n=9) or moderate (n=3) diffuse or multifocal infiltration of lymphocytes. Plasma cells are rarely detectable. In only one mare are neutrophilic granulocytes identified, whereas eosinophilic granulocytes are absent (Tab. 1).

20 of 76 mares investigated show a slight (n=6), moderate (n=10) or severe (n=4) endometritis. In 17 of these cases a

chronic mononuclear endometritis is diagnosed and in three mares an acute exudative purulent endometritis is found.



**Fig. 9:** Exudative ampullitis, 6-month-old foal: Infiltration of neutrophilic granulocytes (red) in the mucosa. (Enzymehistochemistry, CLAE/ chloracetatesterase, magnification 62.5 x)

Exsudative Ampullitis, 6 Monate altes Fohlen: Infiltration von neutrophilen Granulozyten (rot) in der Mukosa. (Enzym-Histochemie, CLAEChloracetatesterase, Vergrößerung 62,5 x)

In one half of these mares with endometritis (n=10) a synchronous inflammation of the uterine tubes is observed. Salpingitis is evident in 2 of 6 mares (33%) with slight, 4 of 10 mares (40%) with moderate and 4 of 4 mares (100%) with signs of a severe mononuclear endometritis.

In 17 of 27 mares salpingitis without endometritis is diagnosed. 17 of 23 mares (75%) with salpingitis are younger than 10 years, whereas 9 of 15 mares (60%) with endometritis are older than 10 years.

**Tab. 1:** Distribution of different inflammatory cells comparing ampulla, isthmus and endometrium (total number of mares investigated: n=76).

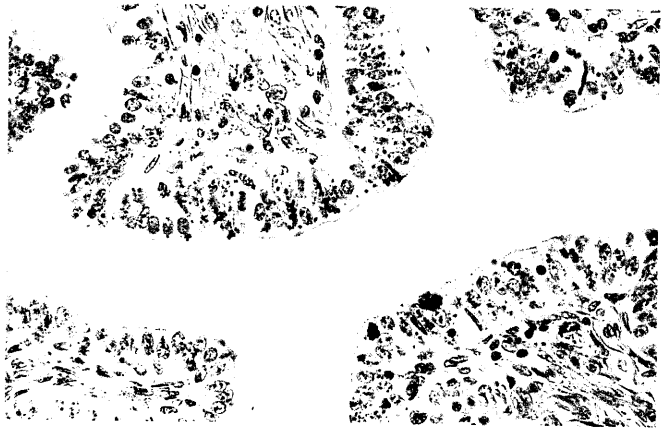
Vergleich der Verteilung verschiedener Entzündungszellen in Ampulla, Isthmus und Endometrium (Gesamtzahl untersuchter Stuten: n=76).

		Ampulla	Isthmus	Endometrium
Infiltration with lymphocytes, plasma cells	slight	16	9	7
	moderate to severe	6	3	10
Infiltration with neutrophilic granulocytes	slight	3	1	-
	moderate to severe	5	-	3
Infiltration with eosinophilic granulocytes	slight	3	-	2
	moderate to severe	-	-	5
No infiltration		49	64	56

Immunohistological staining for chlamydial antigen

In five of twenty mares investigated positive staining for chlamydial antigen is evident and occurs as small spots in

the apical and to a lesser extent in the central or the basal cytoplasm of the epithelial cells (Fig. 10). A non-specific reaction can be excluded because these reaction products are not found in the negative controls. All positive five mares show a multifocal, slight to severe degeneration of ciliated and secretory cells with cytoplasmic eosinophilia. The positive staining is only detectable in several intact epithelial cells and the degenerated eosinophilic cells. The epithelial cells involved have large and hypochromatic nuclei sometimes showing indentations. The staining for chlamydial antigen is more intense in the epithelial cells of the isthmus compared to those of the ampulla.



**Fig. 10:** Isthmus, 21-year-old mare: The positive staining for chlamydial antigen (brown) is predominantly evident in the apical cytoplasm of several epithelial cells. (Immunohistology, Chlamydia psittaci-specific monoclonal antibody, Nomarski-interference-contrast, magnification 125 x)

Isthmus, 21jährige Stute: Chlamydien-Antigen (braun) ist vorwiegend im apikalen Zytoplasma der Epithelzellen darstellbar. (Immunhistologie, Chlamydia psittaci-spezifischer monoklonaler Antikörper, Vergrößerung 125 x)

In only three of these mares is a slight increase of lymphocytes and a few plasma cells of the IgA subtype observed. In two of the mares only, the mucosa of the uterine tubes appears to be markedly changed and a slight infiltration of mononuclear inflammatory cells and moderate to severe degeneration of epithelial cells is evident.

In the endometrium of all of the five mares a slight to moderate endometrosis is observed. Slight to moderate endometritis is diagnosed in only two cases. All mares are classified in the category II b and those with known age (n=4) are older than 15 years.

## Discussion

The examination of the equine uterine tube reveals a high incidence of macroscopic and microscopic lesions. The present study finds infundibular adhesions to be the most common macroscopic lesions in the mare, in agreement with Henry and Vandeplassche (1981) and Satiel et al. (1986). In accordance with our results Henry and Vandeplassche (1981) diagnose no case of infundibular obstruc-

tion. The origin of the infundibular adhesions is unknown but the authors suggest that frequent attacks of colic and peritoneal inflammatory processes lead to the adhesions. The migration of *Strongylus edentatus* seems to be a further cause of infundibular inflammations resulting in adhesions (*Vandeplassche and Henry, 1977*). Furthermore the authors consider that postovulatory hemorrhages from the ovulatory fossa might be responsible.

Comparing the incidence of adhesions in pregnant and non-pregnant mares *Saltiel et al. (1986)* cannot ascertain a marked difference between the two groups indicating that these lesions may not be detrimental to fertility in mares. One of eight pregnant mares in the present study reveals bilateral moderate infundibular adhesions, whereas the fertility of this mare remained intact.

The frequent observation of cysts found in the infundibulum by the current examination is supported by the results of *Saltiel et al. (1986)* who describe „paraovarian cysts“ in 44% of mares. *Mc Entee (1990)* suggested a redefinition of the term „paraovarian cyst“ – incorrectly determining any type of cyst located around the ovary – and use of a specific designation for each type of cyst. The histologic structure as well as the location of a cyst are important for the ascertainment of the origin. Cysts close to the uterine tube or the ovary are usually remnants of the mesonephric or paramesonephric tubules or ducts and are common in most species of domestic animals.

These small congenital cysts frequently occur in the fimbriated portion of the equine salpinx and tend to be multiple. The present study reveals three of eight pregnant mares having small infundibular cysts, thereby proving that infundibular cysts had no effects on fertility. *Blue (1984)* describes the presence of a tubo-ovarian cyst (9cm in diameter) of a young thoroughbred mare causing unilateral obstruction of the uterine tube. This type of cyst is described as a complete fusion of fimbria with the ovary and is extremely rare. The aetiology in the mare remains unknown.

As reported in the study of *Saltiel et al. (1986)* intraepithelial cysts are the most frequent microscopic lesions of the equine salpinx in our material too. In cows the intraepithelial cysts are interpreted as a sequela of hyperestrogenic situations (*Donaldsen, 1969*). *Mc Entee (1990)* observes similar cysts in the fimbria and to a lesser extent in the ampulla of cows on days 9 through to 30 postpartum.

*Odor (1991)* reveals the largest number of intraepithelial cysts in rabbits, especially in the isthmus, with estradiol treatment. The author suggests that intraepithelial cysts derive from a fusion of adjacent ciliated vacuoles. Atypical ciliogenesis may be responsible for the development of ciliated vacuoles. Microtubules, microfilaments and intermediate filaments might be involved in this process too.

The current study reports an atypical immunohistological coexpression of the intermediate filaments desmin and cytokeratin in the epithelial cells. Smooth muscle cells are usually stained for desmin, but the coexpression of desmin and cytokeratin in the epithelial cells is extraordinary. *Wakui (1994)* demonstrates the coexpression of different cytoke-

ratins, vimentin and desmin in the epithelium of the rete testis in the dog.

It might be possible that the ciliated cells of the uterine tube express desmin indicating motility support. The variation of desmin expression in the epithelium therefore can attribute to different cycle stages.

Our results reveal a marked expression of desmin associated with an increase of intraepithelial cysts, but the flattened cells lining the cysts are rarely stained for desmin. Therefore it is doubtful whether desmin should be interpreted as a marker of epithelial damage such as abnormalities of ciliogenic cells. Electron microscopy will be necessary to understand physiological or pathological conditions that lead to the expression of desmin in the epithelial cells.

It is questionable whether pathological significance can be deduced from the frequent occurrence of intraepithelial cysts in mares, especially in those being young or pregnant. If comparable findings in rabbits after estradiol treatment and in cows postpartum are considered an atypical ciliogenesis, likely as a result of hormonal undulation, might lead to intraepithelial cysts.

The incidence of globular masses reported in the uterine tubes of mares varies from 20% to 87% (*van Niekerk and Gerneke, 1966; Liu et al., 1990*). The masses, predominantly characterised as collagen fibres by special stainings, are always located at or near the ampullary-isthmic junction. While *Tsutsumi et al. (1979)* report that the material is connected to the epithelium, *Liu et al. (1990)* find no attachment to the epithelial cells. Two opinions are presented as to the origin of the masses; namely the ovarian or peritoneal origin (*van Niekerk and Gerneke, 1966; Liu et al., 1990*) and the uterine tube mucosal origin (*Tsutsumi et al., 1979*). In our material we can observe neither collagen masses attached to the epithelium nor the evidence of desquamated tubal cells. Therefore we agree with *Liu et al. (1990)* who presume that the collagen derives from the ovary or the peritoneal cavity.

*Aquilar et al. (1997)* find living cells within the intraluminal masses that are predominantly identified as fibroblasts. *Lantz et al. (1998)* attribute the collagen masses with viable fibroblasts to recent ovulation and conclude that connective tissue, drawn in the uterine tube at ovulation, is retained and collagen synthesis continues at least for a few days.

The intraluminal accumulation of large masses of collagen fibres may potentially result in a complete occlusion, causing retention of the ova. Furthermore the collagen might irritate the uterine tube muscles or interfere with the waves of ciliary activity (*Liu et al., 1990*). The authors suspect that the age of a mare might influence the amount of masses since larger masses tend to be present in the uterine tube of some older mares.

The retention of unfertilised oocytes in the mare is unique among domestic animals. These ova originate from previous normal cycle ovulation and persist in the uterine tube for several months (*Betteridge and Mitchell, 1974*). Most frequently the ova are found in the ampullary-isthmic junction and do not have any attachment to the epithelium. Horse embryos therefore bypass the unfertilised oocytes



and enter the uterus. *Freeman et al. (1989a)* suggest that horse embryos initiate transport mechanisms within the uterine tube by secreting a mediator. *Weber et al. (1991)* support the hypothesis that embryonic PGE<sub>2</sub> is involved in the initiation of uterine tube transport in the mare.

To determine the retention of unfertilised oocytes it is of advantage to flush the uterine tube and to search for ova in the flushings whereby lightmicroscopic investigation of paraffin slides only occasionally reveals positive results. This might be an explanation for the negative results in our study concerning the detection of unfertilised oocytes.

The intact uterine tube is determined to be almost free of infiltrating lymphocytes by *Vandeplassche and Henry (1977)*. The present study however states that HE staining alone is inadequate for the detection of the total number of mucosal lymphocytes. With use of specific immunohistological methods for CD 3 lymphocytic surface antigen it is possible to reveal the existence of a certain T lymphocyte population in the epithelium and the stroma even under obviously physiological conditions. Some studies report the nature of „basal“ cells in the epithelium of the uterine tube. *Peters (1986)* identifies the „basal“ cells in human beings as T lymphocytes.

Light focal or diffuse mucosal infiltration of lymphocytes is not uncommon and also frequently observed by *Henry and Vandeplassche (1981)* and *Saltiel et al. (1986)*. These lymphocytes may or may not indicate inflammation. The decision in a certain case is arbitrary. Undoubtedly the infiltration of neutrophils has to be interpreted as an exudative inflammatory process in the equine salpinx.

Our results agree with those of *Vandeplassche and Henry (1977)* describing salpingitis in the mare as mild non-occlusive, infiltrative, or less often exudative, infundibulitis, ampullitis and isthmitis. On the other hand salpingitis in the cow is characterised by masses of connective tissue with extensive cellular infiltration replacing the mucosa and resulting in a complete obstruction of the lumen (*Kessy et al., 1985*). Neither transluminal adhesions of mucosa folds nor obstruction of the lumen is diagnosed in the present study confirming the results of *Henry and Vandeplassche (1981)* who checked the tubal patency by the injection of Bouin's solution and assume that salpingitis in the mare is not as severe, or heals more readily than in cows and sows.

*Allen et al. (1979)* investigate the usefulness of two experimental procedures for the diagnosis of occlusions affecting the equine salpinx. The starch grain test, which is carried out by the injection of a starch suspension onto the ovary and the subsequent recovery of starch from the cervix, is a proven safe and reliable method. Each tube can be investigated separately in contrast to the phenolsulphonphthalein (PSP) test, which implies the deposition of a dye solution into the uterus and its subsequent identification in urine after peritoneal absorption.

The present study finds no predominance of unilateral or bilateral salpingitis and therefore cannot agree with the results of *Henry and Vandeplassche (1981)* who witnessed more frequent occurrences of unilateral salpingitis.

Compared with the isthmus the ampulla is most often involved in inflammatory processes, whereas an isthmitis is

hardly observed. *Henry and Vandeplassche (1981)* who likewise describe an increase of inflammatory alterations from the isthmus to the infundibulum suggest that the infundibulum and the ampulla might be more susceptible to inflammation with regard to their more folded mucosa or that the isthmus recovers more rapidly from inflammation. The authors have no doubt that salpingitis can originate from an endometritis but they also discuss the possibility of salpingitis developing due to an extension of inflammatory processes from the infundibulum and the peritoneal cavity.

*Saltiel et al. (1986)* diagnose slight inflammatory lesions corresponding to the upper segment of the uterine tube and therefore hypothesise that the lesions do not originate from the uterine portion but are the result of disorders, such as non-reproductive diseases in the abdominal cavity, occurring towards the ovarian portion of the salpinx. In mares, the occlusion by a sphincter muscle about the uterine ostium might prevent ascending infections.

The positive immunohistologic reactions using Chlamydia psittaci-antibody is demonstrated for the first time in the equine salpinx. The selective tropism of Chlamydia spp. to cervix and salpinx is suggested as an important circumstance of chlamydial genital infections in several studies in human beings.

*Rank and Sanders (1992)* investigate the pathogenesis of ascending chlamydial infection of the genital tract in guinea pigs. Pathohistological findings are acute or chronic endometritis and salpingitis. Chlamydia are reisolated by culture technique in most of the uterine tubes (78%), while only half of the animals infected (49%) show pathohistological changes.

The presence of plasma cells in three of five mares might be a morphological finding indicating a cellular immune response following primary chlamydial infections as described by *Patton et al. (1989)* in the monkey subcutaneous pocket model. The authors observe early epithelial damages in the secretory and ciliated cells by vacuolisation. Chlamydial inclusions are found in secretory but not in ciliated cells. After tertiary infection especially epithelial eosinophilia, focal areas of epithelial destruction and luminal exudate correlated with mononuclear cell infiltrates are detectable.

This observation agrees with the results of the current study revealing eosinophilia of the epithelium in association with the evidence of chlamydial antigen. Furthermore, infiltration with lymphocytes is found.

By use of special staining (Papanicolaou stain) it can be excluded that the degeneration of the epithelium is related to a metaplastic keratinisation. Electron microscopic studies will have to be carried out to establish whether cytoplasmic eosinophilia is due to chlamydia-induced cytoplasmic degeneration.

Cytoplasmic eosinophilia in the human uterine tube is described as oncocytic metaplasia as well and in the case of association with salpingitis and papillary changes is interpreted as a benign tumour (*Saffos, 1980*). By contrast, no case of papillary changes in the equine salpinx is observed in the present study.

### Correlation between salpingitis and endometritis

Half of the mares with endometritis show signs of salpingitis as well, whereby increasing severity of the endometritis lead to a higher incidence of salpingitis. These observations indicate that the agent causing endometritis can be an important cause of salpingitis as well (Vandeplassche and Henry, 1977). The present study reveals 17 cases of salpingitis in the absence of endometritis supporting the suggestion that an origin of inflammation from the proximal portion of the uterine tube is possible too.

The evidence of chlamydial antigen in the salpinx of the mare is not in any case associated with salpingitis or endometritis. All mares however show a multifocal, cytoplasmic eosinophilia in the uterine tube and a slight to moderate endometrosis.

### Salpingitis and fertility

Salpingitis appears to be slight and only single cases of severe inflammation and damages of epithelial structure are observed.

Tubal infertility of other domestic animals and in women is frequently caused by occlusions of the uterine tube. Salpingitis in the mare, however, is found to be non-occlusive by the methods applied, also in the cases with evidence of chlamydial antigen.

Chlamydia-induced infertility however is based on several pathological processes. Recent studies in monkeys revealed that chlamydial infection causes delayed hypersensitivity which is mediated by the heat-shock-protein (HSP) 60. As a potential antigen this protein leads to immun-induced inflammatory processes of the uterine tube (Patton et al., 1994). Furthermore these pathological mechanisms might directly cause the elimination of the embryo because the heat-shock-protein 60 is one of the first proteins that is evident on the surface of the conceptus.

The epithelium of the uterine tube is not able to realise its function in the case of epithelial degeneration. This epithelial damage, probably induced by chlamydial infection, might imply early embryonic loss in mares as well.

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